

**Does Early BCG in Low Birth weight Newborn less than 2Kg
provide survival advantage in the Newborn Period?**

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In partial fulfillment of the regulations
for the award of the degree of

DM (NEONATOLOGY)

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CERTIFICATE

This is to certify that the dissertation entitled **“Does Early BCG in Low Birth weight Newborn provide survival advantage in the New Born Period?”** is a bonafide work done by **Dr.N.Saravanan** under my guidance and supervision during the period between October 2013 – March 2014 towards the partial fulfillment of requirement for the award of **D.M.(Neonatology)** degree examination to be held in August 2014 by The Tamilnadu Dr.M.G.R. Medical University, Chennai.

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DECLARATION

I solemnly declare that this study title **“Does Early BCG in Low Birth weight Newborn less than 2 Kg provide survival advantage in the Newborn Period?”** was my original work in the Department of Neonatology, Institute of Child Health and Hospital for Children, Egmore, Chennai under the guidance and supervision of **Prof.Dr.J.Kumutha, MD.,DCH.**, Professor & Head of the department, Department of Neonatology, Madras Medical College, Chennai. This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the university requirements for the award of the degree of **D.M. (Neonatology)**

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Dear Dr. N. Saravanan,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Does early BCG <48hrs age in low birth weight Newborn <2kg provide survival advantage in Newborn period"** No.25102013

The following members of Ethics Committee were present in the meeting held on 08.10.2013 conducted at Madras Medical College, Chennai-3.

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INTRODUCTION

Major advances in the management of the preterm low birth weight infants like surfactant, ventilator care, Total Parental Nutrition, central lines, Kangaroo Mother Care, breast milk banking, early feed advancement policy, better asepsis protocols, Antenatal steroids and developmentally oriented supportive care have failed to produce uniformly good outcomes. Sepsis is the major contributor to the mortality. Despite the usage of highly potent antimicrobials, emergence of resistant organisms plague the various newborn care facilities. This has led to a search for alternative therapies to combat sepsis like IVIG and GM-CSF. These have been proven to have doubtful efficacy in recent studies. Against this background BCG vaccine has generated great interest in the western world recently for its non-specific immune modulatory effects in various diseases like Allergy, Cancer, Chronic diseases like Multiple Sclerosis and Diabetes. Historically, since its introduction it had reduced infant mortality rates nonspecifically in certain studies in the 1940's and 1950's. This went unrecognized till now and interest has been rekindled in this age old vaccine.

Recently 2 RCT's in West Africa had found that early BCG vaccine in low

birth weight newborns reduced neonatal mortality significantly by decreasing the incidence of sepsis and pneumonia in these neonates. Based on these findings there is currently a trial going on in Netherlands to study the effect of BCG on infant morbidity. Another study is going on in Australia to determine if it reduces allergy and asthma. A recent large retrospective cohort study done in Australia studying a cohort distributed in many countries concluded that BCG reduced acute lower respiratory tract infections significantly. Molecular studies also support BCG vaccines' ability to enhance immunity (both innate and adaptive) by novel mechanisms. Administration of BCG is delayed for LBW infants in our setting which is similar to Guinea-Bissau. Hence a similar trial to study the effect of early BCG is justified. This low cost intervention if found to be beneficial as certain studies suggest could save a lot of lives and will also have monetary benefits in a resource limited setting.

REVIEW OF LITERATURE

Bacterial sepsis is a major contributor to neonatal mortality and morbidity. 40% of neonatal deaths in India is attributed to sepsis and sepsis related causes^{1,2}. Approximately 20% incidence occurs in various newborn care facilities according to Neonatal Perinatal Database(2003). Neonatal Sepsis incidence in the community is 30 per thousand population³.

The signs and symptoms of sepsis in a neonate are irritability, lethargy, respiratory distress, cyanosis, acidosis, vomiting, poor feeding, unexplained jaundice, bleeding manifestations, changes in body temperature, hypoglycemia or hyperglycemia, pulmonary hypertension, hypotonia, or seizure⁴. The clinical status of neonates can worsen quickly and lead to rapid deterioration. These unique features dictate that the definition of neonatal sepsis remains vague and flexible to physician's interpretation. Neonatal Sepsis includes septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections. Superficial infections like conjunctivitis and oral thrush are not usually included under neonatal sepsis.

The scenario and prevalence in India

The largest perinatal and Neonatal Data base in India 2003 states that neonatal sepsis occurs with a frequency of 30 per

thousand live births. Eighteen NICU units in India which contributed to the Database state that sepsis is one of the leading causes of neonatal mortality³. The gram negative bacteria are a leading cause of sepsis in both inborn and out born newborn care units. Staphylococcus Aureus sepsis occurred in 13.6% cases & Klebsiella accounted for 32.5% of cases in Inborn units. In out born neonatal units pseudomonas was also isolated and it accounted for 10-12% of cases and Klebsiella occurred in 27% of cases.

The major categories of neonatal sepsis

Sepsis in the newborn period is divided into two types. It is based on the timing of occurrence of initial symptoms of infection. Symptoms occurring within the first seventy two hours of life defines early onset sepsis. The respiratory symptoms predominate in early onset sepsis. The organisms are acquired from the mothers reproductive tract and certain maternal factors like PPRM and Chorioamniotitis increase the risk. Neonatal characteristics like birth weight less than 2.5 kg or gestational age less than 37 weeks, meconium staining of amniotic fluid and Apgar less than four at one minute causes an increase in sepsis incidence⁵.

Clinical symptoms occurring beyond seventy two hours of life is late onset sepsis (LOS). It is usually acquired from the hospital or

community . Prematurity, LBW, Ventilator therapy, peripheral& central lines, procedures related to NICU care are certain conditions that predispose the neonate to LOS. It usually manifests as septicemia .Rarely CNS infection can occur^{6,7}.

The immunologically vulnerable preterm Neonate :

Newborn period is a very vulnerable period for sepsis. This is mainly due to defects and immaturity of their immune system. Both innate and adaptive immunity show immaturity. These defects are further amplified in the preterm and low birth weight infants. The compromised nature of immune system in a developing preterm and low birth weight neonate's host defense system allows for bacterial dissemination to extravascular and intravascular sites, including the meninges, lungs and bones.

The innate, immune response is nonspecific and not influenced by prior antigen interaction. Various physical (e.g.cilia,skin⁸, mucus) and biochemical barriers(e.g., lysozyme, gastric acid, surfactant proteins) as well as phagocytic cells (e.g. macrophages and granulocytes) and the plasma factors (e.g., acute-phase proteins ,the complement system, the coagulation cascade, and pattern recognition receptor's like , NOD , Toll)comprise innate immune responses.

The acquired or adaptive immune response is specific. It is comprised of a memory, or an anamnestic response to antigen re-exposure. Acquired immune responses include cell mediated immunity, humoral immunity involving Immunoglobulin production by B lymphocytes and associated factors like Natural killer [NK] cells, cytokines, and interleukins.

The vulnerability of the low birth weight and low gestational age premature infants.

The epidermis of preterm, low birth weight newborn is thin. The stratum corneum is also very thin⁸. Procedures like central and peripheral intravenous catheter insertion adds to the susceptibility of the thin skin to microbial invasion. The bronchial mucosa is vulnerable to infection due to less quantity of protective mucinous glycoprotein in the respiratory secretions¹⁰. This vulnerability to infection is further exacerbated during invasive mechanical ventilation. The ability to cough effectively is also impaired in the low birth weight preterm newborns. The gut is the next most common site of entry of microbes in birth weight newborn less than thirty two weeks gestation age. Adherence of microbes to gut mucosa occurs easily because of low intestinal motility⁹. The stomach hydrochloric acid production is low and the stomach pH is high. The intestinal IgA quantity and the secretory fraction of this

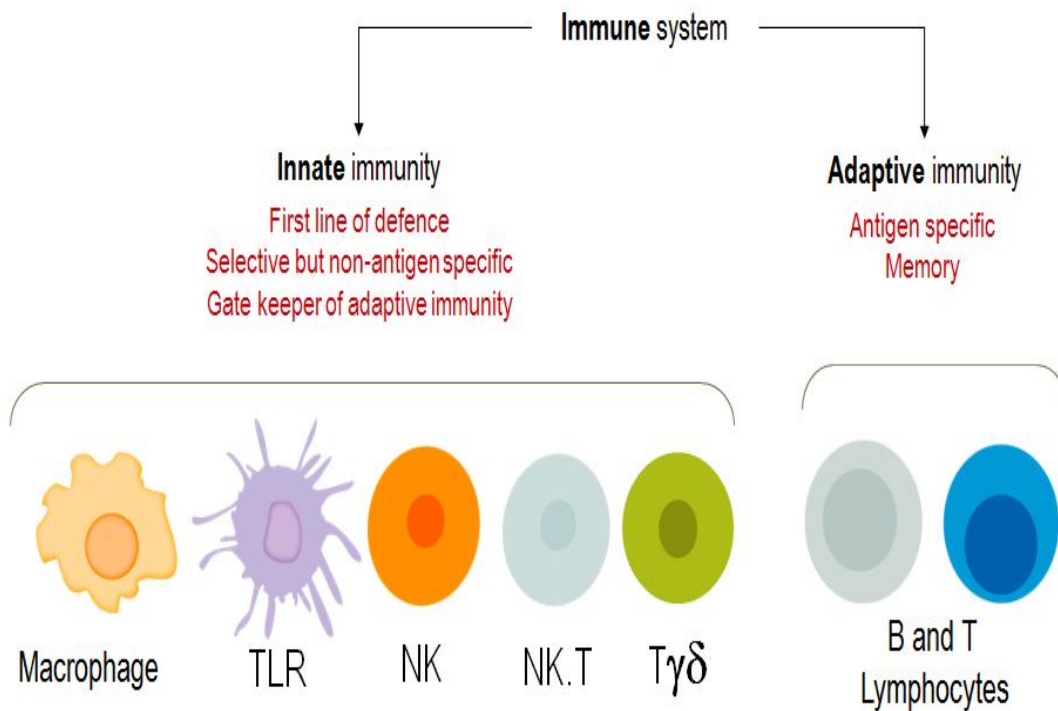
immunoglobulin is especially low in the premature newborn. The integument and the gut mucosa produce low quantity of Defense in a polypeptide molecule. This compound neutralizes bacteria by affecting DNA transcription and increasing the production of immunologically active molecules and cytokines. This compound also acts as chemo attractant to polymorphs and macrophages. They modify the function of the effect or limb of the immune system. Lipopolysaccharide and endotoxin production by bacteria is also inhibited by defensins. The immune response of various antigen presenting cells is also influenced and modified by Defensins^{11,12,13}.

The innate form of immune response in preterm population

Macrophages and the antigen presenting cell have less ability to produce colony stimulating factor and have impairment in identification of microbes by pattern recognizing molecules¹¹. These cells also produce less proinflammatory and immune protective polypeptide molecules called cytokines. The cytokines are necessary to activate the innate immune system^{12,13}. The polymorphs and monocytes have less capacity for phagocytosis and less mobility¹⁴. This causes reduced numbers of these cells at the sites of infection¹⁵. The CD8 cell number is also less in preterm infants and their function is also reduced. The killer cells of the lymphocytic system - the NK cells are reduced in number¹⁶. Anti body independent

cytotoxicity and antibody dependent killing capacity of the cells is less. The complement cascade complex system is also defective¹⁶.

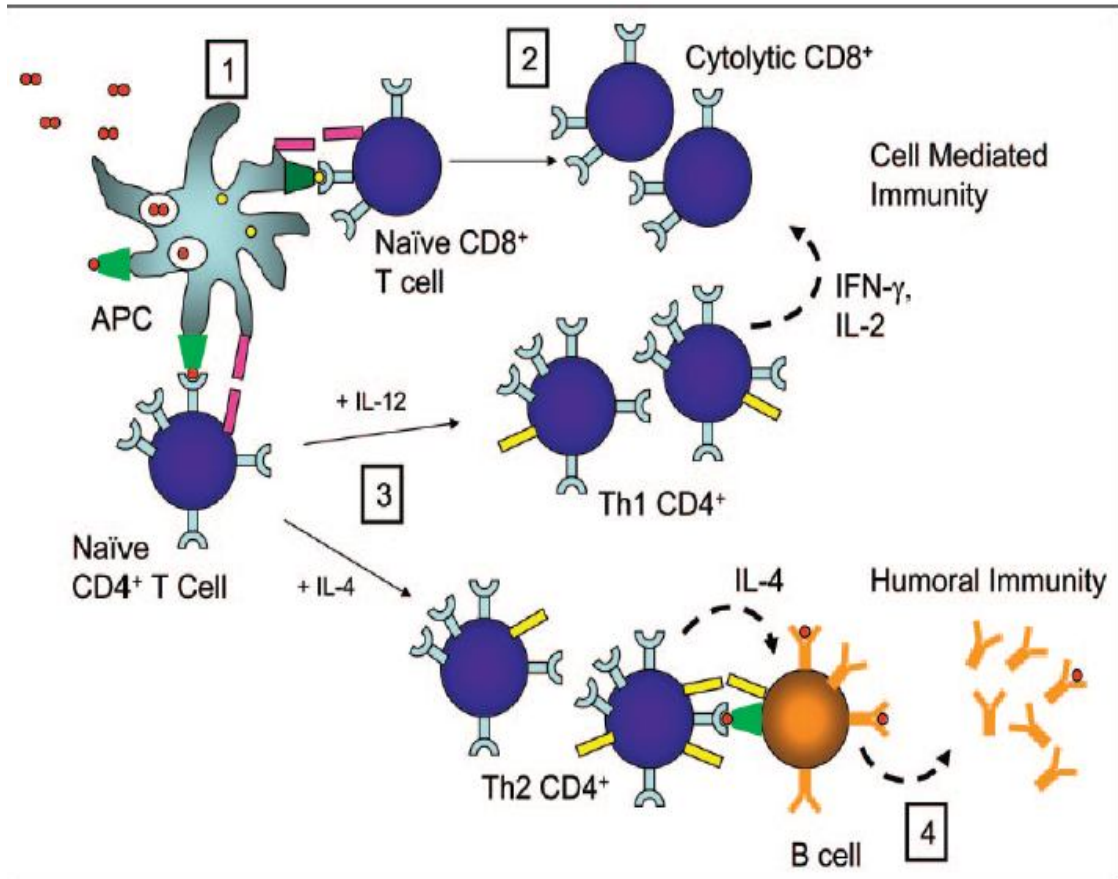
Specific immunity is also affected by reduced effectiveness of the complement system¹⁷. These reduce the quantity of immunogen necessary for adequate production of antibodies. The preterm newborn also has less capacity to secrete gamma interferon. This antiviral substance is secreted by cells which have been invaded by viruses. The macrophages then recognize those cells with viral load and eliminate them^{16,17}.



Adaptiv ehumoral and adaptive cell mediated immunity in preterm neonates.

Acquired specific cell mediated and humoralmediated immune responses in the preterm infant is both immature and also attenuated when compared with a term newborn. Though the quantity of thymic lymphocytes is more in preterm neonate when compared to an older infant, the quality of immune responses is both different and deficient¹⁸.TheThymic helper cell type 1(Th1) response in preterm is defective. The Th1 response is necessary for enhancement of cytotoxic function of lymphocytes and also production of gamma interferon. This form of cell mediated immunity is necessary for destruction of intracellular pathogens in particular¹⁹.The Thymic helper cell type 2 (Th2) polarized response is deficient in comparison to an older term infant. The thymic lymphocytes also have less capacity to multiply to antigenic stimuli^{19,20}.They have less killing activity and capacity against microbes and virally infected cells. The lymphocytes also have less capacity to synthesize interleukins which are important regulatory molecules in immunity. The preterm newborns T cell mediated immune responses like helper T cell induced B-cell stimulation to produce antibodies, Cytotoxic T-cell mediated killing, and recognition of T-cell dependent proteins and immunogens is impaired^{19,20}.Preterms may also have defective B-cell

mediated immune responses according to a recent study but others do not support this.



The full repertoire of antigen recognizing cell receptor on the B-cell lines is acquired only after birth and after 37 weeks gestation. The preterm infant does not have sufficiently enough B-cell receptors to identify the diverse array of microbes in its environment to which it is exposed prematurely^{20,21}. The capacity to produce immunoglobulin across all classes is reduced. IgM is only produced in significant quantity. In addition to these intrinsic defects

the placenta acts as an efficient filter early in gestation and the crossing of class of IgG molecules in to the fetal circulation is impaired^{20,21}. There is a positive correlation between gestational age and antibody levels derived from the mother.

BCG vaccine and Nonspecific immunity

BCG vaccine is one of the oldest vaccines in use in developing countries. It has about 40-50% efficacy in preventing the adult form of tuberculosis according to a Meta-Analysis (Timothy Brewer 2000). At the inception of this vaccine it had been observed that it also reduced infant mortality non specifically. Many studies in America and England in the 1950s^{27,28} showed mortality in childhood in general reduced by 25% with a confidence interval of (0.58-0.92). A retrospective Brazilian study in 1992 showed that BCG reduced lower respiratory tract infection related death by half (Niobey et al)³¹. This study rejuvenated interest in this age old vaccine in the developed nations also. Two randomized controlled trials in Western Africa indicated that BCG improved survival in the low birth weight infant in the newborn period nonspecifically. The mechanism of action remained a puzzle till recently.

It is believed widely that innate immune responses is unchanging. Now evidences have emerged to the contrary which question this age old dogma. Innon-human species like mice and

invertebrate animals and in plants innate immunity has known to have adaptive characteristics^{34,35}. Though this form of immunity, also known as **Trained immunity**^{24,25}, was long known to exist in other species, evidence for its presence in humans is accumulating now. BCG, Freund adjuvant and Muramyl dipeptide are some of the important substances which have been shown to elicit this response in lab experiments^{24,25}. The mechanism of action of BCG seems to be through trained immunity.

In a recent study (Kleinnijenhuis et al) BCG was administered to a sample adult population and Tumor necrosis factor, Interleukin 1 and gamma interferon were studied in this population³². They found an increase of the above mentioned factors against non-specific antigens unrelated to mycobacteria. This effect lasted for greater than ninety days. There was increased levels of CD 11 and TLR on the surface of macrophages³². It was elucidated that epigenetic³³ mechanism like DNA associated Histone protein methylation was involved. Pattern recognition molecule NOD was also involved in this effect. Another study by same investigator was done in T cell and B cell immune deficient mice. In this study BCG protected mice from death due to Candida infection. Mice in control limb died due to disseminated candidiasis^{25,26}. It was found that epigenetic mechanisms like DNA

associated protein methylation and pattern recognition molecule NOD was involved in this effect.

The epigenetic mechanism of action of BCG

The chromosomes of humans contains Histone proteins. Modification and alteration of this DNA associated protein by transfer of methyl and acetyl group carbon moieties causes increased DNA synthesis of inflammatory chemokines and immune regulatory cytokines. The specified group, especially the methyl group, is added to the fourth lysine carbon atom in the histone 3 protein following BCG administration and this acts as an epigenetic mechanism³³. Epigenetic mechanism can be defined as modification of genes by indirect mechanisms rather than by direct action on promoters flanking the gene of interest. This theory was confirmed when it was found that histone methylation of DNA was high in the region of Toll like receptor (TLR) 4 DNA area of macrophages after administration of BCG in volunteers³². TLR are pattern recognizing molecules on the cell surface which play an important role in non-specific immunity to bacteria. The effect lasted till three months after BCG vaccine.

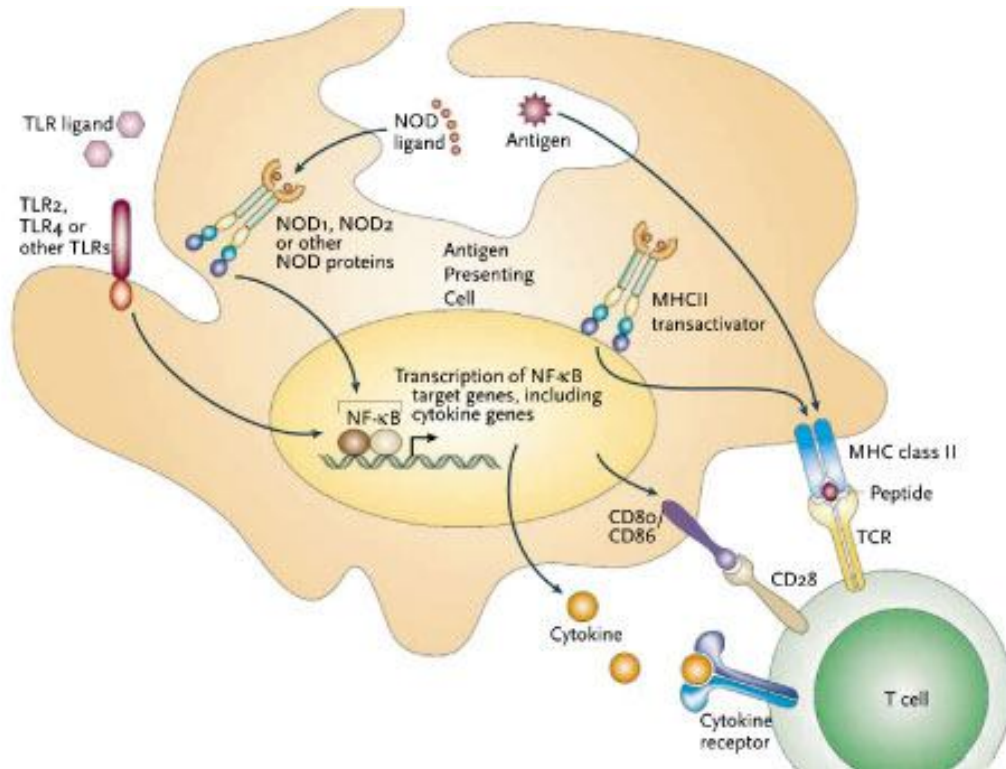
The nucleotide binding oligomer 2 domain receptor (NOD2) mediates the enhancement in innate immune response following BCG

vaccination. Receptor interacting serine /threonine protein kinase 2 (RIP Kinase 2) has also been found to play a role in the BCG mediated effect³². To find the exact mechanism of action of BCG a study was conducted on monocytes in vivo in cell culture. It was found that NOD 2 agonist called the MDP protein caused elevation in synthesis of various immune modulatory cytokines and tumornecrosisfactor after second antigenic exposure. The TLR ligand and agonists like lipopolysaccharide and dead microbes did not have any effect on this immune response. This indicates that NOD mediates the effect of Bacillus calmette guerine on increase in innate immune response³². RIP Kinase 2 blockers diminished this trained immune response and so this compound may also be involved in the enhancement by BCG of trained immune response. Interference with binding of methyl group to DNA associated protein Histone by enzyme inhibitors blocked immune enhancing function of BCG.

Thus increased trimethylation of histone H3 at lysine 4 (H3K4) is the mechanism of action through which BCG enhances non- specific immunity. NOD2 and RIP Kinase 2 act as mediators of this action³².

In confirmation of this hypothesis, H3K4 trimethylation was found to be significantly elevated at the level of cytokine and TLR4 promoters in the circulating monocytes collected more than 3 months after

vaccination with BCG when compared with the quantity before BCG vaccination.



Classical adaptive immunity & Th2 responses to non-specific antigens after BCG

A longitudinal study of BCG Vaccination in Early Childhood was done to assess the effect on Innate and Adaptive immune responses^{34,35} for both specific & non specific antigens by **YennyDjuardi et al.**³⁶ They noted that there was some increase in polyclonal responses to unrelated antigens as assessed by Interferon-gamma and interleukin 5 production when stimulated by PHA (Phytohemagglutinin). The

correlation coefficients between responses to PHA and PPD (purified protein derivative) were strongest at 5 months and one year of Age³⁶. This suggests that BCG can have an effect on cellular immunity to non-mycobacterial antigens also.

In this cohort study the cytokine profiles following BCG vaccination was assessed. Immune response to PPD was assessed before vaccination and at 5 months, 1 year, and 2 years, followed by BCG scar measurement at 4 years.

BCG induced both Th1 and Th2 type responses against PPD at 5 months of age. While Th1 response was sustained, Th2 responses showed a decline over time. BCG scar size strongly correlated with Th2 responses to PPD at 5 months of age. It was also observed that there were no conclusive effects of BCG vaccination on the innate immune responses and no significant increase in IL10 or TNF levels. There was a small increase in general adaptive immune responses to PHA³⁶.

Summary of BCG action mechanism's in enhancing immunity

BCG enhances immune response to unrelated non-mycobacterial antigens in a number of ways.

- 1) Specific and non-specific immunity modification.
- 2) Th2 responses and classic Th1 response.

3) Genetic reprogramming by epigenetic mechanisms affecting innate immunity.

Usually adaptive immune response which is mediated by thymic lymphocytes takes time to be fully functional following heterologous antigen exposure. The trained innate immune responses in contrast takes effect quickly within three days of BCG administration according to some studies (Peter aby et al).

A Randomized trial in Guinea Bissau by **PeterAby and Adam Roth** et al³⁷ tested the effect of BCG vaccine in Low Birth Weight infants as a part of a larger study to assess the effect on infant mortality in study subjects. The study showed that Neonatal mortality ratio was RR 0.52(.33-.82) when BCG was given compared to the control group. There was almost 50% reduction in Neonatal mortality. In this trial the beneficial effect of vaccination occurred within 3 days of intervention³⁷. 9 deaths occurred in the BCG group and 21 deaths in control group. The death Rate fall was due to the reduction in neonatal sepsis and respiratory infections.

The authors concluded that BCG vaccination in low birth weight group was safe. Another study was conducted in Low Birth Weight infants by **Biering Sorensen** et al. It was a randomized trial involving Low-birth-weight children who received BCG vaccination at first health

center visit³⁸. A total of 105 low -birth-weight children coming for first vaccination were recruited. The mortality rate ratio for BCG was 0.17 (95% CI = 0.02-1.35) within 3 days of vaccination, 0.28 (0.06-1.37) till 1 month age, and 0.27 (0.07-0.98) after 2 months of age. The mortality rate ratio was 0.41 (0.14-1.18) (P = 0.098) in infancy. The study concluded that administration of BCG vaccine at first contact may have contributed to the lower mortality in the study population.

Maria-Graciela Hollm-Delgadet al did a study to determine whether BCG vaccination led to a reduction in risk of acute lower respiratory infection (ALRI) among 5 year old children³⁹. It was a retrospective cohort study wherein data was collected from Macro International Demographic and Health Survey and United Nations Children's Fund Multiple Indicator Cluster Survey. A primary cohort of 58021 children from 19 countries (2005–2010) and a secondary cohort of 93301 children in 18 countries (2000–2007) were analyzed. Standardized questionnaires were used during home visits to collect data. Vaccination site was inspected and measurement of general health indicators was made. BCG vaccination was associated with a 17% to 37% reduction in risk of suspected ALRI in both cohorts. Children vaccinated with BCG had a significantly lower risk of suspected ALRI according to the authors ,

but they were not sure whether reduction in underlying risk of Tuberculosis could have contributed to this effect.

BCG vaccination reduction in the development of allergic disease in children was studied in a randomized, prospective, single-blind study by **Steenhuis TJet al** and concluded that there may be a small beneficial effect of BCG and a trend towards less eczema and there was statistically significant less use of allergic medication in eczema patients which may be due to immune modulatory effects of BCG vaccine.

Elizabeth j Anderson et al studied whether strain of BCG vaccine had an influence on specific and non-specific immunity⁴⁰. In Uganda, The influence of BCG vaccine strain on mycobacteria-specific and non-specific immune responses in a prospective cohort of infants in Uganda infants from this study were administered three strains of BCG vaccine – Danish, Russian and Bulgarian. It was found that different strains elicited different immune responses both for tuberculous and non tuberculous antigens. Danish strain was the most immunogenic of the lot and elicited good immune response to specific and non-specific antigens. Adverse reactions were also more in the Danish strain group compared to others. Ninety two percent in the Danish group developed scar at vaccine inoculation site. The Russian strain was

least immunogenic and least adverse effects and scar formation occurred in this group.

The safety and immunogenicity in Preterm newborn infant

Studies by Thayil Sudhin et al and Senghatian et al have established the safety of BCG in preterm babies up to 26 weeks. In the **Thayil Sudhan et al** study⁴², sixty two preterm babies born below 37 weeks of gestation were randomly assigned to two groups. Babies in group 1 were vaccinated early at 34–35 weeks and group 2 were vaccinated late at 38–40 weeks of post-conceptional age. The two groups were similar in terms of mean birth weight (1583 and 1546 gms), gestational age, neonatal medical problems, socio economic status and postnatal weight accretion. The cell mediated immunity to BCG was analyzed using the Mantoux test and the lymphocyte migration inhibition test 6–8 weeks after vaccination. In duration and swelling greater than 5 mm after the Mantoux test was taken as adequate response. There was no significant difference in the tuberculin conversion rates between both Groups (80% v/s 80.7%, respectively). The positive LMIT between groups also was 86.6% and 90.3%, respectively and was statistically insignificant. BCG scarring was similar in both groups (90.0% and 87.1%, respectively).

Another study on tuberculin response in preterm infants after BCG vaccination at birth by **M R Sedaghatian, K Kardouni et al** also showed

similar findings in terms of safety. But, the immunogenicity of BCG was less than preterm infants⁴³. A total number of 101 preterm infants between 26 and 37 weeks gestation received BCG vaccine at birth. They were evaluated at two and four months after BCG vaccination. 32% of these infants had no visible BCG scar. The infants were then administered tuberculin purified protein derivative (PPD). The test was negative in 22 (31%) and there was an induration of 5 mm or less in another 26 (37%) of the infants. Scarring and positive mantoux test was higher in term infants ($p < 0.001$). Of 22 infants with no BCG scar, 19 (86%) had an induration of less or equal to 5 mm. In infants with a positive BCG scar a significantly higher number had an induration of PPD > 5 mm. There were no significant differences between the rate of scarring and positive mantoux test between gestational age and newborn infants born before or after 32 weeks' gestation. The author states that routine BCG vaccination at birth in preterm infants is not indicated and a much larger study has to be performed.⁽³⁰⁾

The current IAPCOI guidelines support BCG vaccine earlier in Low birth weight infants after they are clinically stable.

Thus safety of vaccine is well established in preterms^{42,43} though the immune genicity i.e. specific for mycobacteria has been questioned in babies receiving early BCG. But nonspecific immunity

has not been studied much in babies receiving early BCG. The strain specific study in Uganda and study by **ThayilSudhan et al** indicate it may not be affected by prematurity and is unrelated to scarring. This seems to have immunological basis also as study by **Kleinhaujes et al** propose adaptive features of innate immunity³² for this effect mediated by Histone trimethylation (an epigenetic³³ mechanism). This immune response unlike classical immunity takes very less time to take effect. This short response time is supported by the trial conducted in Western Africa^{37,38}. The effects were observed within 3 days of vaccination. Finally sepsis in preterm low birth weight infants is most lethal in the initial 1st few days of life. Therefore early BCG if it really works is an attractive novel therapy against this background. A significant reduction in Low Birth Weight Infant mortality with a low cost intervention, which has epidemiological, basic science research and clinical evidence backing needs to be explored, which this study intends to do.

JUSTIFICATION OF STUDY

Though advances in various aspects of neonatal care of preterm infants have tended to improve outcomes, neonatal sepsis continues to be a major killer disease. Nosocomial and late onset sepsis a leading cause of mortality. Costly measures like IVIG and GM-CSF have not shown much promise. Against this background BCG vaccine has been shown to have non-specific immunological effects. Recently 2 RCT'S had shown it reduced mortality in low birth weight neonates. Though Indian Academy of Pediatrics Committee on Immunization recommends BCG in low birth weight infants as soon as they are medically stable, most of the babies less than 2000 gm do not receive it during hospitalization, when they are most vulnerable. Therefore there is a need for studying the non-specific effects of BCG in LBW newborn Indian population. Moreover insight about the timing of administration BCG might be gleaned from this study.

HYPOTHESIS

Early BCG vaccination in low birth weight newborns less than 2000 grams soon after birth is useful in reducing mortality during hospitalization due to its Non-specific effects and also may provide survival advantage in the neonatal period.

AIMS AND OBJECTIVES

To learn the effect of early BCG vaccination in low birth weight new born less than 2 Kg on mortality during hospitalization and neonatal survival.

Outcome of the Study

Primary Outcome:

To find if there is a significant reduction in hospital mortality in low birth weight babies less than 2 KG given early BCG (within 48 hours of age) compared with controls.

Secondary Outcome:

- 1) To study the incidence of sepsis, both clinical & blood culture positive sepsis cases in the intervention group and control subjects.
- 2) To compare the Neonatal mortality at follow up in both groups.

METHODOLOGY

Study Title:

“Does Early BCG in Low Birth Weight Newborns Less than 2kg provide survival advantage in newborn period?”

Study centers:

Department of Neonatology, Institute of Child Health & Hospital for Children Egmore (out born unit) and Neonatology Unit, Institute of Obstetrics & Gynecology and Hospital for Women & Children (Inborn unit), Chennai.

Duration of Study:

October 2013 to March 2014

Study Design:

Prospective Randomized controlled trial

Materials and Methods:

Subjects:

Low birth weight neonates less than 2000 grams and less than 48 hrs old admitted to the neonatal units of Institute of Child Health and Institute of Obstetrics & Gynecology. Both tertiary care hospitals in Chennai, India.

Inclusion criteria:

Low birth weight newborns less than 2000 grams admitted in the neonatal unit and less than 48 hours of age.

Exclusion criteria:

- 1) Infants with major anomalies (CDH, TEF, Duct dependent CHD, Intestinal obstruction, Major skeletal dysplasia).
- 2) Very sick neonates (perfusion defect in spite of inotropes, respiratory failure and DIVC at time of recruitment).
- 3) Babies born to Hepatitis B positive mother (immunoglobulin would be given to child) and HIV positive mothers.
- 4) Denial of consent

Sample Size:

The baseline mortality from previous year 2013 was around 33% for the population which we intended to study. A sample size of 277 in each limb was arrived at for an effect size of 30% reduction (based on the West African study) for an alpha error of .05 and a power of 80%. The total sample size was 554.

Randomization:

Computer generated random numbers generated for 6 stratified groups separately according to weight category and sex were used during randomization.

The stratified weight based groups were;

- 1) less than 1000gms
- 2)1000-1499gms
- 3)1500-1999gms

Each group was divided in to male and female to ensure equal distribution among sex and weight categories in the intervention and control limbs.

The intervention

Each day eligible babies in both inborn and out born units were identified and recorded on a register. The babies who fulfilled the inclusion criteria were enrolled after obtaining the necessary consent from any one of the parents. The babies who had the exclusion criteria were excluded.

The babies were randomized to intervention or control limb by opening opaque sealed envelopes with the respective weight and sex strata printed on the sides of the envelope. 6 packets of envelopes for each stratification category were used.

A ten Dose vial of BCG vaccine (Serum Institute India) was reconstituted and intradermal BCG vaccination was given to the intervention group in the left shoulder region at deltoid insertion. 0.05ml of vaccine was given using 26 gauge attached needle insulin

syringe. A wheal of 6mm-8mm was produced in all vaccinated children. These babies were monitored for any acute reactions.

Controls received no BCG till discharge.

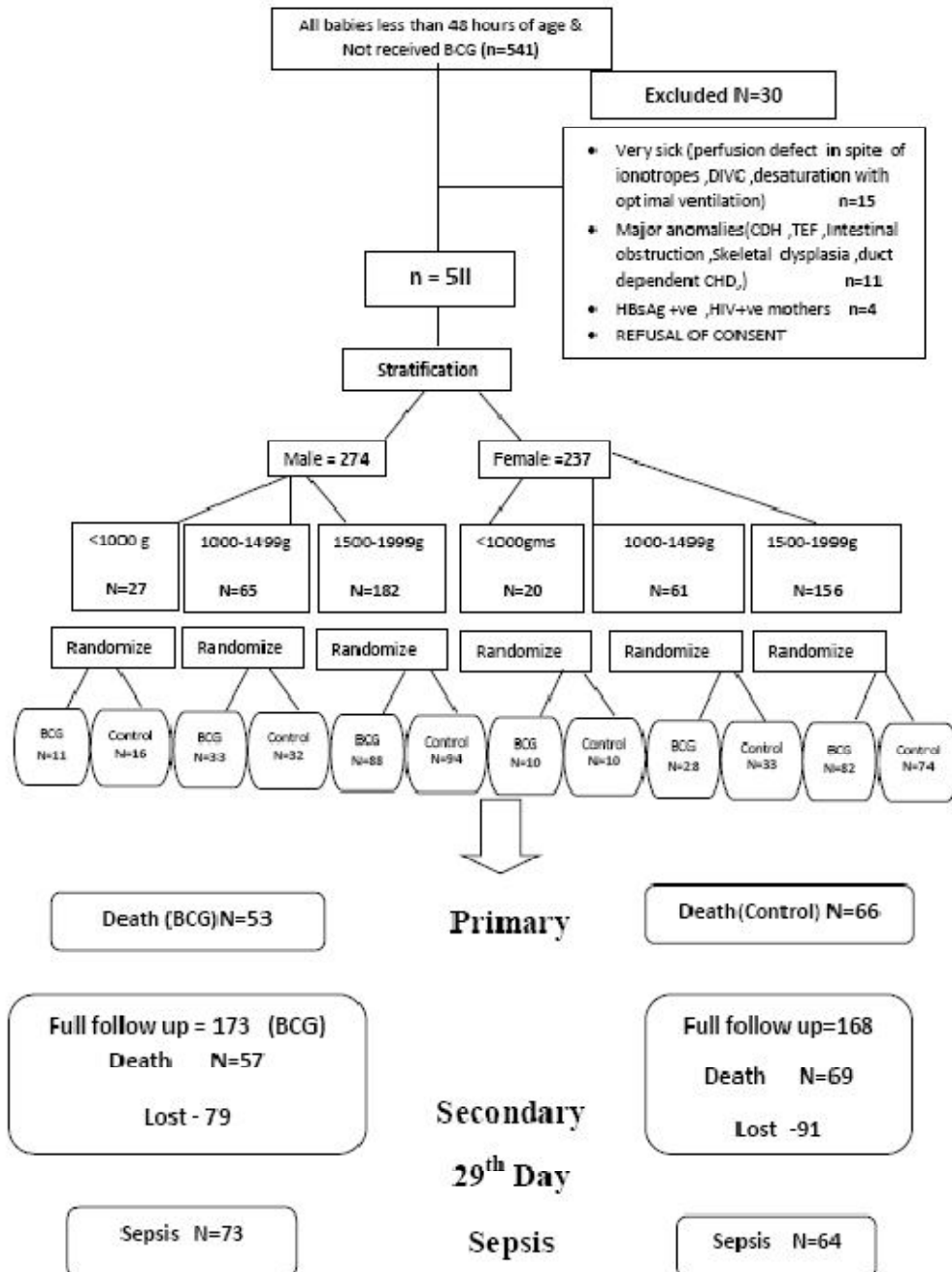
Data Collection:

The data was filled in individual case forms. A separate diary was maintained for 29th day follow up enquiry. The mothers were given a printed card on which the study number and vaccine details were mentioned to avoid revaccination and to facilitate follow-up. Telephone tracking was done to check the well-being of the babies. Death or discharge from Hospital was the **primary end point**. Live status on 29th day of life was these **secondary end point**. The collection of necessary information regarding secondary outcomes completed the case recording. The information was transferred to a validated excel spreadsheet.

Statistical Analysis:

All statistical analysis was performed according to intention to treat principle. SPSS software version 18 for Windows was used in analysis, standard statistical tests were employed. Categorical variables were analyzed using chi square test. Continuous variables were analyzed using student t test. Statistical significance was considered at a p value of less than 0.05.

CONSORT DIAGRAM FLOW DIAGRAM



RESULTS

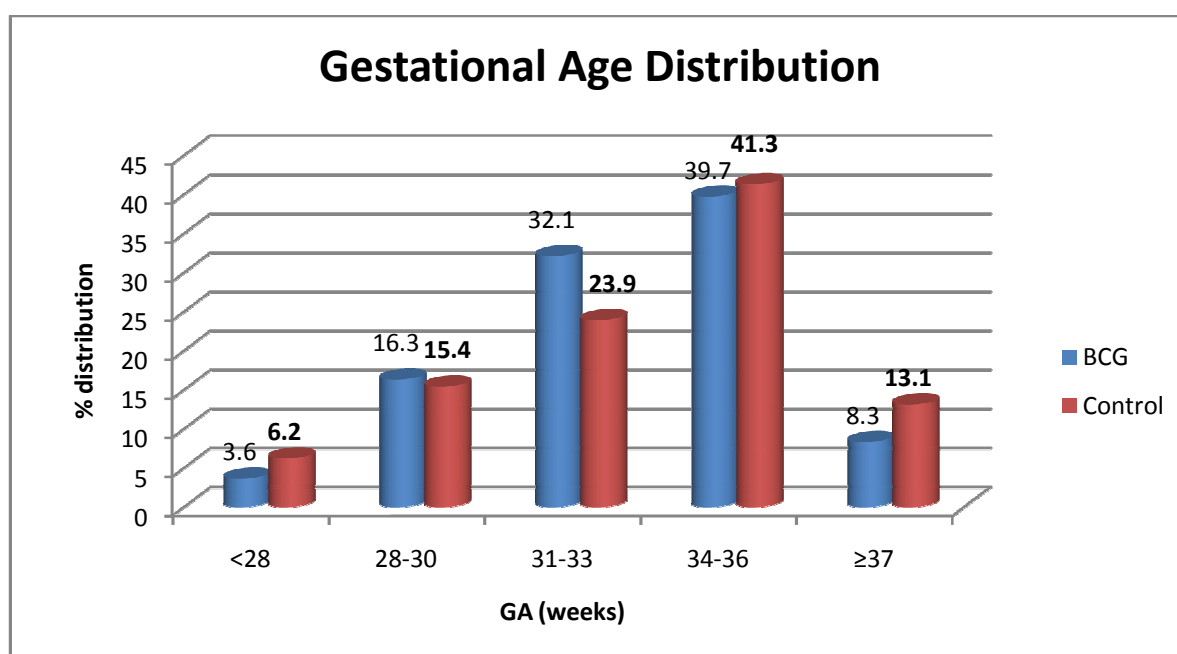
A total of 511 babies were recruited into the study as against the calculated sample size of 554. The total admission of eligible babies during the period was less than expected. 252 babies were randomized to intervention group and 259 to control group.

252 babies received BCG.

138 male babies and 136 female babies received BCG. 136 male babies and 101 female infants got allocated to control group.

Table : 1 Gestational age distribution

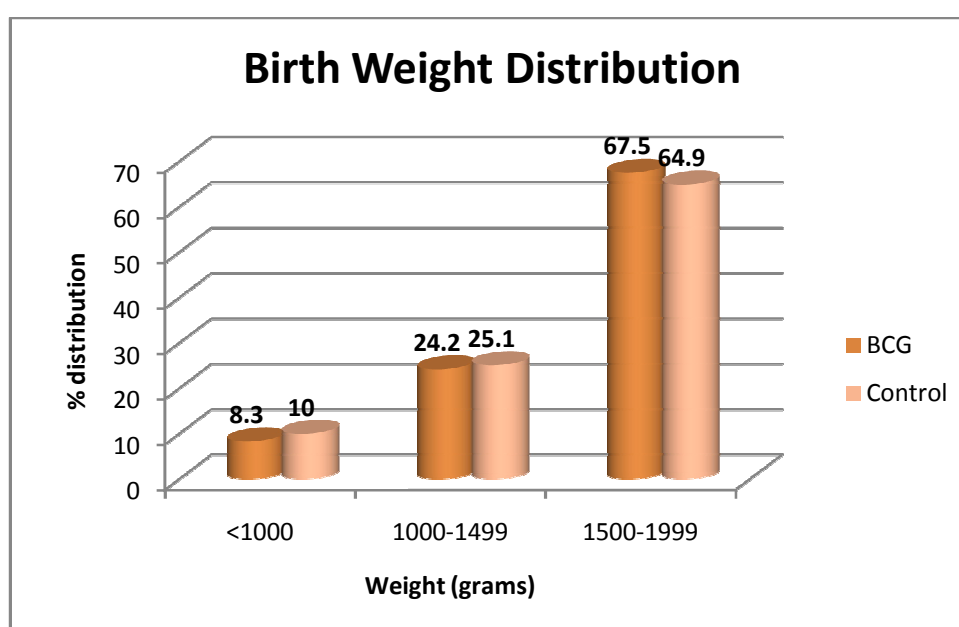
Gestational age (weeks)	BCG(252)	Control(259)	P value
Less than 28	9(3.6)	16(6.2)	0.103
28-30	41(16.3)	40(15.4)	0.103
31-33	81(32.1)	62(23.9)	0.103
34-36	100(39.7)	107(41.3)	0.103
≥37	21(8.3)	34(13.1)	0.103



Most of the babies included in the study were in the gestational age group of 34-36 weeks (40.5%). Only 4.9% of the study population was less than 28 weeks of gestational age. The distribution of babies in the BCG and control groups across the various strata of gestational age was comparable.

Table : 2 Birth weight distribution

Birth wt(grams)	BCG	Control	P value
<1000	21(8.3)	26(10)	0.750
1000-1499	61(24.2)	65(25.1)	0.750
1500-1999	170(67.5)	168(64.9)	0.750



Nearly 66% of the babies had birth weights in the 1500-1999 g range. Extremely low birth weight babies accounted for 9.2% of the study population. Comparable distribution was observed in various categories of birth weight in both intervention and control groups.

Table : 3 Baseline Maternal Characteristics

Characteristic	BCG	Control	P value
Maternal age (yrs)	24.2 ± 2.1	23.6 ± 2.4	0.167
Primi	152(60.3)	156(60.2)	0.654
Diabetes / GDM	31(12.3)	32(12.4)	0.399
Cardiac Disease	4(1.6)	8(3.1)	
Hypertension	19(7.5)	8(3.1)	
Seizure disorder	5(2.0)	5(1.9)	
Thyroid disorder	11(4.4)	7(2.9)	
Anemia	13(5.2)	11(4.3)	
Renal	1(0.4)	1(0.4)	

Maternal factors like age, parity, anemia, hypertension, heart disease, diabetes were comparable in both the groups.

Table : 4 Perinatal factors

Characteristics	BCG	CONTROL	P value
Risk of EOS (%)	148(58.7)	150(57.9)	0.923
PROM>24 hours (%)	13(5.2)	21(8.1)	0.097
Multiple gestation (%)	58(23.0)	59(22.8)	0.198
Mode of delivery L.S.C.S Normal vaginal Instrumental	112(44.4) 133(52.8) 7(2.8)	110(42.5) 142(54.8) 7(2.9)	0.897
AN steroids Full course Partial	48(19.0) 86(34.1)	54(20.8) 71(27.4)	0.258
Meconium stained liquor	16(6.3)	11(4.2)	0.387
Antibiotics(IAP)	31(12.3)	25(9.7)	0.414
PIH Eclampsia APH	72(28.6) 19(7.5) 9(3.6)	56(21.6) 16(6.2) 14(5.4)	0.420

All the perinatal baseline parameters were comparable in both the groups.

Table : 5 Baseline Characteristics

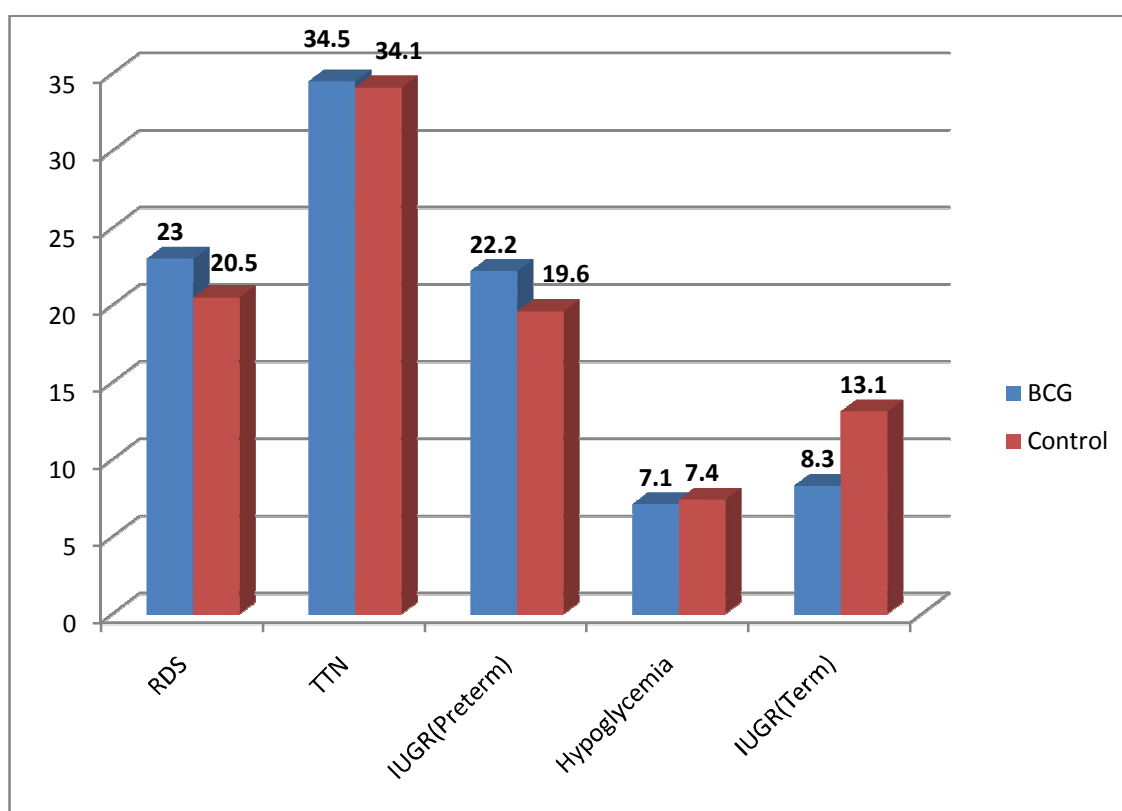
CHRACTERISTIC	BCG (252)	CONTROL (259)	P value
Mean Gestation age (week)	32.98± 2.6	33.10± 2.7	0.670
Mean birth wt (grams)	1591.55±334	1570.56± 359	0.495
Mean Age recruitment hr	10.39 ±5.26	10.31±6.06	0.874
Male (%)	138(54.8)	136(52.5)	0.673
Inborn (%)	203(80.6)	196(75.7)	0.220

The important base line characteristics like mean gestational age, mean birth weight, mean age of recruitment and gender distribution were statistically comparable in both groups.

Table : 6 Neonatal morbidity Data

Characteristic	BCG	Control	P value
RDS	58(23)	53(20.5)	0.544
TTN	87(34.5)	88(34.1)	0.454
Preterm/ IUGR	56(22.2)	51(19.6)	0.671
Term /IUGR	21(8.3)	34(13.1)	0.103
Hypoglycemia	18(7.10)	19(7.4)	0.609

Neonatal morbidity on admission evenly distributed. Term / IUGR accounted for 10.7% of the study population.



The Neonatal baseline morbidity was distributed similarly in both groups.

Table : 7 Treatment Related Factors

Characteristic	BCG	Control	P value
NICU Level-2	97(38.5) 155(61.5)	103(39.8) 156(60.2)	0.838
Surfactant	47(18.7)	39(15.1)	0.334
Invasive Ventilation	67(27.6)	63(24.3)	0.699
CPAP	92(36.7)	75(29.0)	0.079
I.V fluids	134(53.2)	137(52.9)	0.330
Antibiotics	139 (55.2)	148 (57.1)	0.296
Ventilation days <3Days 3-7Days >7Days	18(7.1) 35(13.9) 14(5.6)	25(9.7) 30(11.6) 8(3.1)	0.336
FFP	36(14.3)	34(13.1)	0.801
Inotropes	80(31.7)	77(29.7)	0.691

No significant difference was there in treatment received by both groups.

Table : 8 Primary Outcome

Characteristic	BCG	Control	P value
Death in hospital	53(21)	66(25.5)	0.278
Discharge	199(79)	193(74.5)	0.278

25% in the control group and 21% in the BCG group succumbed in hospital before discharge. Though the percentage was less in BCG group, this was not statistically significant.

Table : 9 Primary outcome inferential statistics

Characteristic	BCG	Control	Odds ratio	Confidence interval	P value
Death in Hospital	53(21)	66(25.5)	0.78	0.52-1.18	0.278

Though the odds ratio appears to favor BCG group, the wide confidence interval (0.52-1.18) with the range of value extending beyond one suggests that this is not statistically significant. The p value also is 0.278.

Table : 10 Secondary outcome Incidence of Sepsis

Characteristic	BCG	Control	P value
Culture positive sepsis	12(4.8)	18(6.9)	0.143
Screen positive	61(24.2)	46(17.8)	0.143

The babies were monitored for occurrence of sepsis during their hospital stay. 73 neonates in BCG group and 64 neonates in control group underwent septic workup when they developed clinical features suggestive of sepsis. 12 babies in the BCG group and 18 babies in the control group had culture proven sepsis. Nearly 24% in BCG group and 18% in control group were screen positive but culture negative. Though statistically insignificant, culture positive sepsis was less in BCG group when compared with the control group.

Table 11

Characteristic	BCG	Control	P value
29 th Day tracking	120	102	0.08
Post discharged death (up to 28 days)	4	3	0.826
Survived till 28 days	116	99	0.826

On 29th day follow-up 120 babies in BCG group and 102 babies in control group were tracked. 4 babies in the intervention group and 3 babies in the control group died post discharge. 79 in the BCG group and 91 in the control group could not be tracked in spite of earnest efforts. The lost for follow-up percentage was 33%, but this was at secondary end point. There was no significant difference in post discharge mortality among the two groups.

Table 12

Organism	BCG	Control	P value
E.coli	9(3.6)	12(4.6)	0.735
Klebsiella	2(.8)	4(1.5)	0.735
Staph.aureus	1(.4)	1(.4)	0.735
Pseudomonas	0	1(.4)	0.735

The organisms isolated in culture positive cases were E.coli, Klebsiella, Staph aureus and Pseudomonas. E.coli was the dominant isolate in both the groups. There was no significant difference in the type of organisms grown in culture between groups.

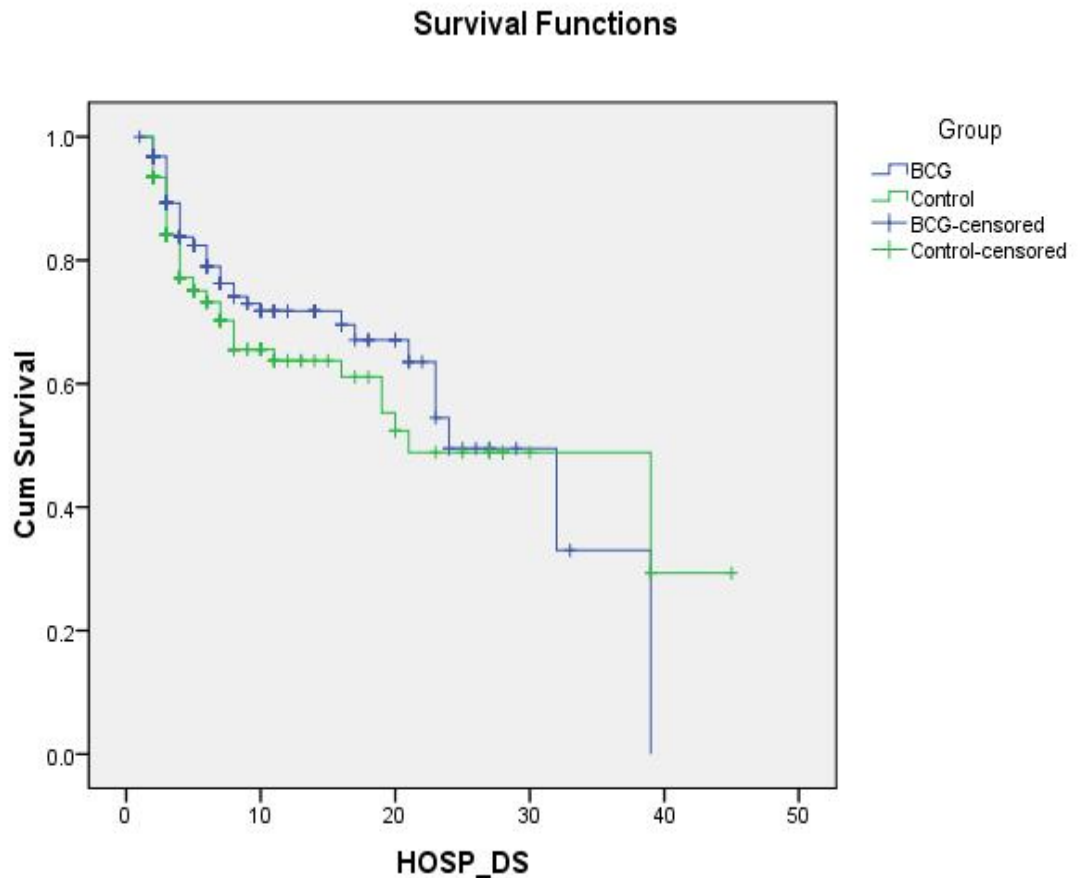
Table : 13 Duration of hospitalization

Characteristic	BCG	Control	P value
Hospitalization days	7.12 ± 6.8	6.42 ± 7.3	0.272

The mean duration of hospitalization was similar in both groups - 7.12 days in BCG group against 6.42 days in control group.

Survival statistics

The survival function shows a small favorable benefit of BCG on survival During 1st 20 days of hospitalization but was not significant.



Overall Comparisons

	Chi-Square	df	Sig.
Tarone-Ware	3.640	1	.056

The vector of trend weights is -1, 1. This is the default.

Table : 14 Sepsis related mortality

Sepsis	BCG (n=73)	Control(n=64)	P value
Death	32(43.8)	36(56.2)	0.201
Discharge	41(56.2)	28(43.8)	0.201

Sepsis related mortality was not statistically different in both the groups.

DISCUSSION

This single center study to analyze the non-specific immunological effects of BCG in reduction of in hospital mortality in low birth weight neonates, the incidence of sepsis and provision of survival advantage in the newborn period is first of this kind to be conducted in India.

The present study came up with the following results; The trial shows a slight reduction in Hospital mortality in the BCG group 21% as against 25.5% in the control group. But it is not statistically significant. The secondary outcomes like sepsis and mortality in neonatal period showed no significant difference. In fact 29% of neonates had sepsis in BCG group against 24.7% in controls. Sepsis related mortality was also not significantly different in both the groups though it was less (43.8%) in BCG group compared to (56.2%) control group . The mean duration of hospitalization was 7.12 days in BCG group. It was marginally longer than control group (6.42 days) but was not significant.

The West African study by **Peter Aby** et al had evaluated Infant Mortality in two groups (Early BCG and Routine practice) which was their primary outcome. They failed to show a significant result. Though there was 17% reduction in infant mortality in early BCG it did not reach significance. (IMR in early BCG 10.2% and control 12.4%) A general reduction in infant mortality in general population was cited as one of the

reasons for the negative result. In secondary analysis, neonatal mortality ratio was studied and they found a significant relative risk reduction of 48% in neonates.

The other similar study by **Beiring Sorensen et al** Bandimin Guinea-Bissau,³⁸ is discussed. A Total of 105 LBW (<2500 gm) coming for first vaccination were randomized to receive bacillus BCG immediately or later. (Current practice) The Mortality RR for BCG was 0.17 (CI = 0.02-1.35) within 3 days of enrollment, 0.28 (0.06-1.37) in the 1st month, and 0.27 (0.07-0.98) after 2 months of age. The Mortality RR reduced to 0.41 (0.14-1.18) (P = 0.098) in infancy. The study population was neonates less than 2500 gm compared to ours where we included babies weighing less than 2000 gm. Their study population was recruited from out patients while we recruited only inpatients. Though there was a reduction in mortality to an extent of 72% in their study the confidence intervals were wide making it statistically insignificant. Sepsis was the leading cause of mortality in both African studies which is also the major determinant of mortality in our study. The strains used in the **Beiring Sorensen et al** study was also Danish.

The possible reasons for failing to detect a significant reduction in mortality; There is a beneficial effect but it is less than the expected effect size which this study was designed to detect. The explanations for the lack of beneficial effect might be the following.

- a) The strain used in this study was the Russian which may be weakly immunogenic and may not elicit the non-specific beneficial effect of BCG in study population.
- b) The young preterm infants may not have sufficient immune response to the BCG vaccine in comparison to older neonates
- c) The ethnicity and genetics of the study population.

BCG strain

The West African study by **Peter Aaby et al** used the Danish strain of vaccine and their study showed a relative risk reduction 0.55(0.34-0.89). The Russian strain has been found to be less immunogenic in a study from Uganda. In that Study by **Elizabeth J Anderson et al**⁴⁰ Infants were administered three different strains of BCG - Danish 1331, Russian and Bulgarian. Immune regulatory molecule levels were assessed after a period of one year to both specific and non-specific antigens. The strains differed in their immunogenicity significantly. Scar formation and adverse effects were significantly different among the strains. BCG of Danish strain was most immunogenic. The Russian strain was found to be least immunogenic for specific and non-specific antigen.

Prematurity and poor immune response to vaccine

The second reason could be the poor immunogenicity of the BCG vaccine in preterm Babies. The study by **Senghatian et al** shows significant reduction in immunogenicity assessed by scar, BCG nodule and Mantoux reactivity between term and preterm neonates⁴³. Adverse events and scar frequency was significantly less in this group. Some studies suggest that specific immune responses of preterm neonates to BCG vaccine may be impaired. The lack of sufficient non-specific immune response in preterm population itself could be one of the reasons for the negative result in the present study. But **ThayilSudhin et al** have found the vaccine to be equally immunogenic in preterm babies based on Mantoux, LMIT and BCG scar.⁴²

The third reason could be the ethnicity and genetics of the study population. There is no study comparing non-specific effects of BCG in various races. Studies on the specific immune response of BCG indicate it varies with the ethnicity and geography. The most controversial aspect of BCG is the variable efficacy found in different clinical trials, which appears to depend on geography. Trials conducted in the UK have consistently shown a protective effect of 60 to 80%, but those conducted elsewhere have shown only 50% Protective effect, and efficacy appears to fall the closer one gets to the equator. BCG was shown to have no

protective efficacy against pulmonary tuberculosis in the ICMR study in the South Indian population.

A 1994 systematic review found that the BCG reduces the risk of getting TB by 50% (Timothy et al). The differences in effectiveness, depends on several factors such as genetic differences in the populations, changes in environment and exposure to other bacterial infections.

Though, molecular study in adults by **Johanneke Kleinnijenhuis et al** showed that BCG vaccination in Healthy adult volunteers led to a four- to seven fold increase in the production of Interferon gamma and to a twofold enhanced release of monocyte derived cytokines, such as TNF and IL-1 β , in response to unrelated bacterial and fungal pathogens. Molecular studies in newborns and children to assess this Non-specific immunity have not been done so far.

The possibility of inadequate sample size

The effect size of 30% might be too optimistic and a larger sample size may be required. The baseline mortality for the intended study group in 2012 year was around 33%. The extra mural unit had a higher mortality though the number of admissions in the select category was 1/3rd of the total study population of interest. As there were no previous such studies in India and the West African studies had shown almost 50% reduction in mortality a conservative 30% effect

size was chosen to Calculate the sample size. It was also assumed that 2/3rd of cases would be recruited from intramural unit and 1/3rd from extramural unit based on previous year Data. There was a reduction in number of cases from the out-born unit and only 19% were recruited from it. There was also a reduction in mortality in general decreasing the baseline mortality to 23.3% against the expected 33%. Therefore a larger sample size may be needed to detect a statistical difference between both groups.

The final reason for the outcome maybe that the non- specific effects of the vaccine may be too insignificant in study population to benefit clinically. But this study does not have adequate power to substantiate that claim. A larger trial with a smaller effect size, larger sample is required

Strengths of the study.

It is a Randomized Control Trial Demonstrated the feasibility of early administration of BCG in low birth weight neonates. Safety of early BCG has been established fairly well as we did not record any adverse side effects following vaccine.

Limitations of the study

The calculated sample size of 554 in total could not be achieved due to a general decrease in the number of admissions both in inborn and out-born unit and 511 cases were recruited in total. There was 33.6% loss to follow up for the secondary outcome of Neonatal Mortality. The BCG strain used was Russian strain, while the other studies in West Africa used Danish strain. This may be a confounding factor as the study in Uganda felt Russian strain was less immunogenic. Efforts were made to procure the Danish strain but could not be procured.

CONCLUSION

Early BCG administered to low birth neonates less than 2000 grams within 48 hours of life did not confer any significant survival advantage to these neonates. Early BCG did not show reduction in the incidence of infections in low birth weight babies.

Early BCG failed to reduce mortality in the neonatal period significantly. Further larger studies are required to prove or disprove the hypothesis, maybe with a lesser effect size and more immunogenic vaccine strain.

Future directions for Research

The future studies must target a smaller effect size with a larger sample size using the different strains of BCG vaccine to get a definitive answer on the benefit of BCG in the study population.

The cytokine profiles may also be assessed in future studies for elucidating Immunological effects in this low birth weight population.

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PROFORMA

Name	age	sex	
Place of delivery	DOB	B.wt	
Maternal details			
Mode of delivery	LMP	EDD	
GA			
Antenatal h/o			
Prom			
Tocolysis			
AN steroids			
Delivery details			
Resuscitation details	apgar	1mt	5mt
Sepsis risk score (AIIMS)			
perinatal h/o			
Reason for referral(out born)			
Mode of transport			
Support on arrival			
Condition on arrival	temp		vitals
Presenting symptoms & signs			

Anthropometry	HC	CC	length
wt			
NBS score		ga	
Admitting diagnosis			admission wt
DOA	time		age at adm
Admitting hospital			
Hemodynamic stability			
Major malformation			
HIV/HepB status mother			
BCG group assigned	early		age in hrs
control			
Course during hospitalization			
Final diagnosis			
Primary outcome			
Secondary outcome(sepsis)			
29 th day outcome			
Hospital management details			
Surfactant			
Ventilation			
Antibiotics& duration			
Anticonvulsants			
Ionotropes			

Methyl xanthines

Exchange transfusion

Phototherapy

Partial exchange

Blood component

FFP

PRBC

Platelet

IVF

PPN

ABBREVIATIONS

AN steroidAntenatal steroids

BCG Bacillus CalmetteGuerine

IAPCOI IndianAcademy of Pediatrics Committee on

Immunization

EOS Early Onset Sepsis

LOS Late Onset Sepsis

TLR Toll Like Receptor

IFN Interferron

CD Cluster of Differentiation

Th1 Thymic Helper Cell type 1 response

B cell B Lymphocyte

NOD2 Nucleotide binding oligomer 2 domain receptor

RIP kinase 2 Receptor interacting serine protein kinase

H3K4 Histone H3 at lysine 4

NK cell Natural Killer Cell

PHA Phytohemagglutinin

IL-10 Interleukin-10

TNF Tumor Necrosis Factor

INFORMATION SHEET

- We are conducting a study on Does Early BCG Vaccination <48hrs age for Low Birth Weight Neonates <2kg provide survival advantage in newborn period. All low birth weight newborns less than 2kg presenting within 48 hrs to ICH and IOG are eligible to be considered for participation. For this your approval and consent may be valuable to us.
- Need for study-It is to find if giving BCG early in Low Birth Weight infants less than 2kg within 48 hrs reduces the high mortality observed in this population with a low cost intervention with minimal adverse events
- BCG is a common vaccine given to most babies soon after birth to prevent Tuberculosis. It has been given for over half a century and is one of the safest vaccines. Preterm and low birth weight babies especially less than 2kg have increased chance of serious infection and even death when compared with normal weight babies in the 1st 28 days of life, this is due to their decreased resistance to infection. Usually these low birth weight babies receive BCG vaccine later when they weigh 2 kg, this policy has no strict scientific backing though it has become a routine. Recently it has been found in a few promising studies that BCG vaccine when given early increases survival in Low birth weight babies in the 1st 28 days of life by increasing resistance to infections other than Tuberculosis. The purpose of present study is to explore this advantage. In this study the BCG vaccine shall be given to babies less than 2kg earlier within 48hrs or as per usual routine when they weigh 2kg. The babies enrolled shall have equal chance of receiving BCG vaccine early or late. It has been found in other studies that giving BCG earlier does not increase the side effects of vaccine in the low birth weight babies. Your participation shall be purely voluntary.
- The main purpose of the study is to assess if there is an increased survival in the study population who have received early BCG vaccine than those receiving it after 7 days. The eligible and enrolled babies will have equal chance of either receiving the vaccine early or late.
- We are selecting the babies based on eligibility criteria and other treatments shall be uniform according to hospital protocol
- Intervention- Low Birth Weight infants less than 2 kg shall be randomized to receive BCG vaccine as an injection into the skin over the left shoulder within 48 hrs of age or receive it later as controls (when babies achieve a wt of 2kg) usually after 1 week
- Adverse events- Hypotension, respiratory depression, skin rash, fever, rarely disseminated infection
- Safety measures- babies receiving early vaccination shall be monitored for 24 hrs for any of the complications under cardiorespiratory monitor and appropriate interventions shall be prompt and they shall be followed up till 28 days for rare events

- The participation is voluntary and after your consent, you are free to withdraw from the study when ever you desire and there shall be no compulsion involved, other treatments and benefits shall not be affected by your decision not to participate
- Follow up period will be till 28 days
- The privacy of the patients in the research will be maintained throughout the study. In case of any publication or presentation resulting from research no personally identifiable information shall be shared
- Taking part in the study is voluntary, you are free to participate in the study or withdraw at any time, your withdrawal shall not result in any loss of benefits which you are otherwise entitled to. The results of the study will be intimated to you at the end of the study period.

Signature of the investigator

signature of the parent

Date

chennai

CONSENT FORM

I Ms/Mr. _____ M/O//F/O, B/O _____

Sex _____ Hosp. No. _____ admitted in the Neonatal ward of ICH & IOG,

Egmore was explained to by the doctor that my baby is diagnosed to have Low Birth Weight and This condition predisposes the baby to increased risk of mortality. I am willing for my child to be enrolled in the "Does Early BCG < 48hrs age in Low Birth Weight < 2kg provide survival advantage in newborn period" study. The doctors have explained to me the nature and the purpose of the trial. I have given my consent only after completely understanding the details that were explained to me, that the BCG vaccine shall be given by an injection on the left shoulder in to the skin within 48hrs of birth if the baby is selected by random process and have read the information sheet for the study. I am also aware that the baby would receive the vaccine later if not selected after 7 days. I am willing for my baby to be enrolled in this study without any ones compulsion and I am fully aware that I can withdraw from the trial at any time during the study. I have given consent for administering Early BCG as per the study protocol. I .The adverse effects (hypotension, respiratory depression skin rash & dissemination) were explained to me. I have given this consent to be enrolled in this study with my full consciousness.

Signature of Investigator

Signature of Parent

ஆராய்ச்சியின் தகவல் தெரிவித்தல் தாள்

- ❖ நாங்கள் “குறை எடை பச்சிளங்குழந்தைகளுக்கு சீக்கிரம் 48 மணி நேரத்திற்குள், BCG தடுப்பு ஊசி 28 நாட்களுக்குள் போடுவதன் மூலம் பொதுவாக நன்மையளித்து உயிர் பிழைக்கும் வாய்ப்பை கூட்ட உதவும்” என்ற ஆராய்ச்சியை நடத்துகிறோம்.
- ❖ அரசு குழந்தைகள் நல மருத்துவமனை எழும்பூர் மற்றும் அரசு மகபேறு மருத்துவமனை எழும்பூர், சென்னையில் உள்ளோயாளியாக அனுமதிக்கப்படும் 2கிலோ எடைக்கு குறைவான மற்றும் 48மணி நேரத்திற்கு குறைவான வயதுள்ள குழந்தைகள் இந்த ஆராய்ச்சியில் பங்குபெற தகுதியானவர்கள். பங்கேற்கும் ஒருதரப்பினருக்கு BCG தடுப்பூசி சீக்கிரமாக போடப்படும், மறுதரப்பினருக்கு 2KG எடை ஏறியபின் ஒரு வாரம் தள்ளிப்போடப்படும். BCG தடுப்பூசி போடுவதால் பக்க விளைவுகள் மிகவும் குறைவு. அவை மூச்சு திணறல், இரத்த ஓட்டக் குறைப்பாடு, தோலில் தடிப்பு, புண், நோய் பரவுதல் ஆகும். இதற்காக உங்கள் குழந்தை முழு கண்காணிப்பில் 24 மணிநேரம் இருப்பதால் உடனடி சிகிச்சை பெறுவர்.
- ❖ இதற்கு உங்கள் ஒப்புதலும் சம்மதமும் எங்களுக்கு தேவை.
- ❖ இந்த ஆராய்ச்சியின் குறிக்கோள்:
இதில் பங்கு ஏற்கும் மற்றும் ஆராய்ச்சியின்படி சிகிச்சையளிக்கப்படும் குழந்தைகளின் உயிர் பிழைக்கும் வாய்ப்பை கூட்டுகின்றதா என்று அறிவதாகும். 28 நாட்கள் முடிவில் பங்கேற்கும் குழந்தைகள் காணப்படுவர்.

- ❖ குழந்தைகள் ஆராய்சி நியமனங்களுக்கு ஏற்ப தேர்வு செய்யப்படுவார்கள் மற்ற சிகிச்சைகள் அனைத்தும் மற்ற குழந்தைகளுக்கு அளிப்பதற்கு சமமாக இருக்கும். மருத்துவமனை விதிகளுக்கு ஏற்ப சமமாக இருக்கும். ஆராய்ச்சியில் பங்கேற்பது. உங்கள் முழு சம்மதம் தெரிவித்த பிறகு நடைபெறும் இதிலிருந்து விலக விரும்பினால் எப்போது வேண்டுமானாலும் செய்யலாம். இதனால் உங்கள் குழந்தைக்கு தரப்படும் மற்ற சிகிச்சை மற்றும் சலுகைகள் பாதிக்கப்படாது.
- ❖ ஆராய்ச்சியில் பங்குபெறும் குழந்தைகளின் தனிப்பட்ட விவரங்கள் காக்கப்படும்.
- ❖ ஆராய்ச்சியில் பங்குபெறுவது உங்கள் முழு விருப்பத்தின் பேரிலேயே ஆகும். நீங்கள் எப்பொழுது வேண்டுமானாலும் ஆராய்ச்சியிலிருந்து விலகிக் கொள்ளலாம். இதனால் உங்கள் குழந்தைக்கு சாதாரணமாக கிடைக்க கூடிய சலுகைகள் மற்றும் சிகிச்சை எந்த விதத்திலும் பாதிக்கப்படாது.
- ❖ ஆராய்ச்சியின் முடிவில் வரும் விடைகள் மற்றும் விவரங்கள் உங்களுக்கு தெரியப்படுத்தப்படும்.

ஆராய்சியாளர்

பெற்றோர்

தேதி:

சென்னை

ஆராய்சிக்கு ஒப்புதல்/சம்மதம் தெரிவித்தல்

நான் திரு/திருமதி..... மற்றும்

ஆண்/பெண் குழந்தை மற்றும் எண்ணில் அரசு குழந்தைகள் நல மருத்துவமனை (ICH) அரசு மகப்பேறு மருத்துவமனை எழும்பூரில் அனுமதிக்கப்பட்ட நோயாளியின் தாய்/ தந்தை ஆவேன்.

எனக்கு என் குழந்தை குறை எடை உள்ளதாக விளக்கப்பட்டது.

இந்த நிலை அதன் உயிர் பிரிவதற்கான வாய்ப்பை அதிகரிக்கும் என்றும் விளக்கப்பட்டது. BCG தடுப்பு ஊசி சீக்கிரமாக, 48 மணி நேரத்திற்குள் போடுவதால் இதைக் குறைக்கலாமா என்பது ஆராய்ச்சியின் குறிக்கோள் என்று அறிந்தேன்.

நான் என் குழந்தையை “குறை எடை குழந்தைகளுக்கு சீக்கிரம் 48 மணி நேரத்திற்குள், BCG தடுப்பு ஊசி போடுவதன் மூலம் பொதுவாக நன்மை அதிகரித்து பிழைக்கும் வாய்ப்பை அதிகரிக்க உதவும்” என்ற ஆராய்ச்சியில் பங்கு பெற முழு சம்மதத்துடன் ஒப்புதல் அளிக்கிறேன்.

மருத்துவர்கள் இந்த ஆராய்ச்சியின் குறிக்கோள் மற்றும் நடைமுறை பற்றி எனக்கு விளக்கினார்கள். ஆராய்ச்சியின் தகவல் தான் முழுமையாகப்படித்து புரிந்த பின்னர் இந்த ஆராய்ச்சியில் பங்கு பெற எனது சம்மதத்தை அளிக்கிறேன்.

நான் எனது சம்மதத்தை இந்த ஆராய்ச்சியைப் பற்றி நன்கு புரிந்த பின்னரே அளிக்கிறேன்.

என்னை இந்த ஆராய்ச்சியில் பங்குபெற யாரும் வற்புருத்தவில்லை. என் சுயவிருப்பத்தின் பேரிலேயே இதில் என் குழந்தை பங்குபெற சம்மதம் அளிக்கிறேன்.

இந்த ஆராய்சியிலிந்து நான் என் குழந்தையை எப்போழுது வேண்டுமானாலும் விலக்கமுடியும் என்று அறிவேன்.

நான் என் சம்மதத்தை ஆராய்சியின் விதிமுறைகளுக்கு ஏற்ப BCG தடுப்பு ஊசிபோடப்படும் என்று அறிவேன். ஒருதரப்பினருக்கு இந்த ஊசி 2Kg எடை ஏறியபின்னர் ஒரு வாரம் கழித்தப்பின்னரே போடப்படும் என்று அறிவேன்.

BCG போடுவதால் நேரக்கூடும் பக்கவிளைவுகள் மிகவும் குறைவு என்றாலும் அதன் விவரங்கள் (ரத்த ஓட்ட சீறினமை, மூச்சு திணறல், சருமத்தில் சிகப்பு தடிப்பு, ஊசிபோட்ட இடத்தில் புண், நெறிக்கட்டுதல், கிருமி பரவுதல்) எனக்கு விளக்கப்பட்டன. இதற்கான எச்சரிக்கை நடவடிக்கைகள் பற்றியும் அறிவேன்.

நான் இந்த ஆராய்சியில் பங்குபெற என் முழு சுயசிந்தனையுடன் சம்மதம் அளிக்கிறேன்.

பெஜ்ஜேர்

brvKamal u	3	2	2	20, 12, 13	20, 12, 13	25, 12, 13	6	6	1	1	1	1	2	7	8	2	2	1	7 5 0	5	8	3	2	0	1	1	1	3	2	2	3	2	2	2	2	1	3	3	4	1	5	5	5	2	1	1	3	2	2	4	1	3	5	2	2	1	
brvSugant hi	3	2	1	15, 12, 13	15, 12, 13	24, 12, 13	1	2	6	1	7	1	1	7	8	2	2	0	1 5 0	5	8	4	1	6	1	1	1	3	2	1	3	1	2	2	2	2	1	4	2	4	1	5	2	9	2	1	1	3	2	2	1	1	2	9	2	2	1
brvDhamal axmi	3	7	2	14, 12, 13	14, 12, 13	18, 12, 13	2	4	6	1	7	1	2	7	8	1	2	0	1 5 0	5	8	1	3	0	1	1	1	5	2	1	3	2	2	2	2	2	1	3	4	1	5	9	5	2	1	1	3	2	2	4	1	3	4	2	2	1	
brvAela	3	3	2	21, 11, 14	21, 11, 14	26, 11, 14	1	6	4	5	1	1	7	9	1	2	0	1 5 0	5	8	1	1	0	1	1	1	5	2	1	3	2	2	2	2	2	1	3	4	1	5	9	5	2	1	1	3	2	2	4	1	3	5	2	2	1		
brvSmith a	3	6	1	3,1 2,1 3	3,1 2,1 3	5,1 2,1 3	1	2	6	1	7	1	2	7	8	4	2	0	1 9 0	5	8	1	2	0	1	1	1	3	2	2	3	2	2	2	2	1	3	4	1	5	5	5	2	1	1	3	2	2	1	1	3	2	2	1			
brvAmudh a	3	1	1	31, 10, 13	31, 10, 13	22, 11, 13	1	0	6	4	5	1	1	7	8	2	1	1	1 5 0	5	8	5	2	2	1	1	1	2	2	2	3	1	2	2	1	1	3	2	1	1	3	2	9	2	1	1	3	2	1	2	1	2	2	2	1		
brvKaduk a	3	1	1	19, 11, 13	19, 11, 13	30, 11, 13	1	2	6	1	7	1	2	6	7	2	2	0	1 5 0	5	8	3	2	3	1	1	1	2	2	2	3	1	2	2	1	1	3	2	4	1	5	3	9	2	1	1	3	2	2	1	1	3	1	2	2	1	
brvMadhi mu	3	2	1	12, 14	12, 14	18, 2,1 4	2	6	4	4	1	1	6	7	1	2	1	1 7 4 5	5	8	1	1	2	1	1	1	2	2	2	3	1	2	2	2	2	1	4	2	2	1	5	2	8	2	1	3	3	2	2	1	1	1	1	7	2	2	1
brvDevaki	3	6	2	11, 12, 13	11, 12, 13	14, 12, 13	1	2	6	4	5	1	1	8	9	1	2	0	1 5 0	5	8	1	3	0	1	1	1	5	2	1	3	2	2	2	2	2	1	3	4	1	5		5	2	1	1	3	2	1	4	1	3	3	2	2	1	
brvVijaya umi	3	4	2	15, 13	15, 13	29, 11, 13	6	6	4	2	1	1	7	8	1	2	0	1 3 5 6	1	5	1	1	2	1	1	1	2	2	1	3	1	2	2	2	2	1	4	2	4	1	5	2	3	2	1	1	3	2	1	5	1	2	1	4	2	2	1
brvVijaya umi?	3	4	2	15, 11, 13	15, 11, 13	22, 11, 13	6	6	4	2	1	1	7	9	1	2	0	1 6 7 0	1	8	1	1	0	1	1	1	5	2	1	3	2	2	2	2	2	1	3	4	1	5	9	9	2	1	1	3	2	1	4	1	3	7	2	2	1		
brvChinna ngammal	3	4	1	30, 11, 13	30, 11, 13	6,1 11, 2,1	2	6	2	4	1	1	8	9	1	2	0	1 9 0	5	8	1	3	0	1	1	1	5	2	1	3	2	2	2	2	2	1	3	4	1	5	9	5	2	1	1	3	2	2	1	1	3	7	2	2	1		
brvMunee vmar?	3	9	2	2,1 2,1 3	2,1 2,1 3	7,1 2,1 3	4	6	1	4	1	2	8	9	1	2	1	1 9 9 0	1	8	1	3	0	1	1	1	5	2	1	3	2	2	2	2	2	1	3	4	1	5		5	2	1	1	3	2	2	4	1	3	5	2	2	1		
brvMunee vmar?	3	9	2	2,1 2,1 3	2,1 2,1 3	6,1 2,1 3	4	6	1	4	1	2	7	8	1	2	0	1 9 9 0	1	8	1	3	0	1	1	1	5	2	1	3	2	2	2	2	2	1	3	4	1	5		5	2	1	1	3	2	1	4	1	3	4	2	2	1		
brvSajath al	3	4	2	16, 12, 13	16, 12, 13	19, 12, 13	1	0	6	4	4	1	1	8	9	1	2	0	1 4 1 1	1	8	1	2	0	1	1	1	3	2	2	3	2	2	2	2	2	1	3	4	1	5		5	2	1	1	3	2	1	5	1	3	3	2	2	1	
brvSajath al	3	4	2	16, 12, 13	16, 12, 13	19, 12, 13	1	0	6	4	4	1	1	8	9	1	2	0	1 5 0	1	8	1	2	0	1	1	1	3	2	2	3	2	2	2	2	2	1	3	4	1	5		5	2	1	1	3	2	1	4	1	3	3	2	2	1	
brvSripri u	3	2	9	11, 12, 13	11, 12, 13	16, 12, 13	1	2	6	1	7	1	1	5	7	2	2	1	1 5 4	5	8	5	1	5	1	1	1	2	2	2	3	1	2	2	1	1	4	2	1	1	5	2	3	2	1	2	3	2	1	2	1	1	2	4	1	2	1
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brvKathu	3	3	1	6,1 2,1 3	6,1 2,1 3	17, 12, 13	2	6	1	1	1	1	4	7	1	2	0	1 5 8	5	8	1	3	2	1	1	1	2	2	2	3	2	2	2	2	1	3	3	4	1	5	3	2	2	1	1	3	2	1	1	1	2	1	2	2	1		
brvAmbik amb-vil	3	2	1	19, 11, 13	19, 11, 13	24, 11, 13	1	2	6	2	4	1	2	8	9	2	2	0	1 8 5	1	8	2	2	3	1	1	1	2	2	2	3	2	2	2	2	1	2	4	1	5	3	9	2	1	1	3	2	1	1	1	3	5	2	2	1		
brvAmbik amb-2	3	2	1	19, 11, 13	19, 11, 13	24, 11, 13	1	2	6	2	4	1	2	7	8	2	2	0	1 8 0	1	8	2	3	2	1	1	1	2	2	2	3	2	2	2	2	1	3	4	1	5	3	9	2	1	1	3	2	1	1	1	3	5	2	2	1		
brvVictori u	3	6	1	4,1 2,1 3	4,1 2,1 3	6,1 2,1 3	6	6	4	5	1	2	7	8	2	2	0	1 9 0	5	8	3	2	0	1	1	1	5	2	1	3	2	2	2	2	2	1	3	4	1	5		5	2	1	1	3	2	1	1	1	3	2	2	2	1		
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brvShakila	3	5	2	4,1 2,1 3	4,1 2,1 3	9,1 2,1 3	8	6	4	5	1	1	7	8	1	2	0	1 7 3	5	3	1	3	0	1	1	1	3	2	2	3	2	2	2	2	2	1	3	4	1	5		5	2	1	1	3	2	1	4	1	3	5	2	2	1		
brvSharan l	3	7	2	4,1 2,1 3	4,1 2,1 3	7,1 2,1 3	3	6	1	1	1	1	8	9	1	2	0	1 8 0	5	8	1	3	0	1	1	1	5	2	1	3	2	2	2	2	2	1	3	4	1		5	2	1	1	3	2	1	4	1	3	3	2	2	1			
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brvShalini l	3	7	1	2,1 2,1 3	2,1 2,1 3	5,1 2,1 3	5	6	5	4																																															

br/athu	3	4	2	19, 11, 13	19, 11, 13	22, 12, 13	2	6	1	7	1	2	7	8	2	2	1	7	0	5	8	2	3	1	1	1	1	2	2	1	3	2	2	2	2	1	3	4	1	5	3	5	2	1	1	3	2	1	4	1	3	3	2	2	1	
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bby Pramma1	3	4	2	17, 12, 13	17, 12, 13	19, 12, 13	2	6	5	4	1	2	8	9	2	2	1	1	1	1	1	0	1	1	1	1	3	2	2	3	2	2	2	2	2	1	3	4	1	5		5	2	1	1	3	2	2	4	1	3	2	2	2	1	
bby Pramma2	3	4	2	17, 12, 13	17, 12, 13	19, 12, 13	2	6	5	4	1	2	8	9	2	2	0	1	1	1	1	0	1	1	1	1	3	2	2	3	2	2	2	2	2	1	3	4	1	5		5	2	1	1	3	2	2	4	1	3	2	2	2	1	
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br/Kohini	3	4	1	6, 1, 14	6, 1, 14	10, 1, 14	8	3	1	5	2	2	6	8	2	1	1	8	2	3	6	2	2	1	1	1	1	2	3	1	1	1	1	1	1	4	1	3	3	1	2	7	2	3	1	3	1	1	1	1	2	2	4	1	1	2
br/Anurady a	3	0	1	10, 1, 4	10, 1, 4	15, 1, 1, 1	8	5	1	7	2	1	6	7	2	2	0	5	8	2	3	5	2	1	1	1	1	2	3	1	1	1	1	1	4	1	2	3	1	2	7	2	3	3	3	2	2	2	2	1	4	1	1	2		
br/Satruva	2	8	1	12, 1, 4	12, 1, 4	16, 1, 1, 1	2	5	4	5	4	1	4	6	4	1	5	5	1	1	2	6	2	1	1	1	1	2	3	1	1	1	1	2	1	3	1	2	1	5	1	1	2	3	1	3	2	2	3	4	2	2	2			
br/Chitra	2	6	1	1, 1, 4	1, 1, 4	13, 1, 1, 1	2	5	1	1	4	2	4	7	4	2	0	5	8	1	2	6	2	1	1	1	1	2	3	1	1	1	1	1	4	1	2	3	1	2	7	2	3	1	3	2	1	3	2	6	1	1	2			
br/Santha nalam	2	6	2	16, 1, 1, 4	16, 1, 1, 4	19, 1, 1, 1	2	4	1	1	2	2	5	7	1	2	7	5	8	1	3	3	1	2	1	2	2	3	1	2	2	2	1	1	4	1	2	1	5	6	2	2	2	1	3	2	1	6	2	2	3	1	2	2		
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[illegible]

tr/hansir	3	3	2	15, 1.1 4	15, 1.1 4	22, 1.1 4	6	6	1	1	1	1	7	8	1	2	1	7 8 0	5	8	1	3	4	1	1	1	1	1	2	3	1	1	1	1	1	1	1	3	1	1	1	5	1	1	2	1	1	3	2	1	4	1	3	7	1	2	1
tr/indhu msh	3	4	2	12, 1.1 4	12, 1.1 4	21, 1.1 4	6	6	1	7	1	1	8	9	1	2	1	9 8 8	5	8	1	3	0	1	1	1	3	2	2	3	2	2	2	2	2	2	2	2	3	4	1	5	9	9	2	1	1	3	2	1	4	1	3	6	2	2	1
tr/karish m	3	6	1	9.1, 14	9.1, 14	11, 1.1 4	8	6	4	5	1	2	7	8	1	2	5	9 6 5	5	8	1	1	0	1	1	1	3	2	2	3	2	2	2	2	2	2	1	3	4	1	5	5	2	1	1	3	2	2	1	1	3	2	2	2	1		
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tr/amm a	2	7	1	1.1 2.1 3	1.1 2.1 3	4.1 2.1 3	4	6	1	7	1	1	5	7	2	2	0	7 7	5	8	3	2	4	2	1	1	1	1	2	3	1	1	1	2	1	3	1	2	1	5	1	1	2	1	1	3	2	2	3	2	3	3	1	2			
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h/o/Karpuz ml1	3	1	1	16, 1, 4	16, 1, 4	23, 1, 4	5	6	3	4	1	2	7	8	1	2	0	2	8	1	2	0	1	1	1	3	2	1	3	2	2	2	2	2	2	1	3	4	1	5	9	5	2	1	1	3	2	2	4	1	3	7	2	2	1	
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h/o/Swamy a	3	3	2	16, 1, 4	16, 1, 4	23, 1, 4	7	6	4	5	1	1	7	8	1	2	5	5	8	1	2	2	1	1	1	2	2	2	2	3	2	2	2	2	1	2	1	3	4	1	5	3	9	2	1	1	3	2	1	4	1	3	5	2	2	1
h/o/Chitra ramil	3	5	1	17, 1, 4	17, 1, 4	23, 1, 4	6	6	4	4	1	1	7	8	4	2	0	1	8	1	2	0	1	1	1	3	2	1	3	2	1	3	2	2	2	2	1	3	4	1	5	9	5	2	1	1	3	2	1	1	1	3	5	2	2	1
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h/o/Sivara njani	3	6	2	3, 1, 14	3, 1, 14	9, 1, 14	8	6	1	7	1	1	7	8	4	2	0	1	5	6	1	3	0	1	1	2	3	2	1	3	2	2	2	2	1	3	3	4	1	5	9	5	2	1	1	3	2	2	4	1	3	6	2	2	1	
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h/o/Rohini	3	3	1	6, 1, 14	6, 1, 14	10, 1, 14	3	4	5	2	2	2	7	8	1	2	0	1	5	5	1	3	6	2	1	1	1	1	2	3	1	1	1	1	1	3	1	2	1	5	1	5	2	3	1	3	2	1	1	2	3	4	1	2	2	1
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